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*Rapid communications*

# Infusion of the 5-hydroxytryptamine agonists RU24969 and TFMPP into the paraventricular nucleus of the hypothalamus causes hypophagia

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**Abstract.** The 5-$HT_{1B}$ agonist RU24969 when given either systemically (1 mg/kg SC) or by infusion (0.5, 1.0, 2.0 μg) into the region of the paraventricular nucleus of the hypothalamus caused dose-dependent hypophagia in rats previously deprived of food for 18 h. Similar results were obtained at the above dosages of 1-[3-(trifluoromethyl) phenyl] piperazine (TFMPP), which acts on 5-$HT_{1B}$ and possibly also on 5-$HT_{1C}$ receptors. Neither drug significantly affected locomotion following central administration. Food intake was significantly decreased when the 5-$HT_{1A}$ agonist 8-OH-DPAT was given systemically (1 mg/kg SC) to rats previously deprived of food but was unaffected when 8-OH-DPAT (1 μg) was infused into the paraventricular nucleus of both food-deprived and free feeding rats. Therefore, hypophagia occurs when hypothalamic 5-$HT_{1B}$ (and possibly 5-$HT_{1C}$) but not 5-$HT_{1A}$ receptors are activated.

**Key words:** 5-$HT_{1A}$ receptors – 5-$HT_{1B}$ receptors – 5-$HT_{1C}$ receptors – RU24969 – TFMPP – Feeding – 8-OH-DPAT – Hypothalamus – Paraventricular nucleus – Rat

Food intake is decreased by systemic injection of the 5-hydroxytryptamine (5-HT) precursor 5-hydroxytryptophan and the 5-HT releaser fenfluramine (reviewed Sugrue 1987). This effect is mediated (at least in part) by 5-HT receptors in the paraventricular nucleus (PVN) of the hypothalamus, as infusing 5-HT or norfenfluramine into this site causes hypophagia (Shor-Posner et al. 1986). However, it is not clear which 5-HT receptors are involved. Radioligand studies have revealed at least three types of 5-$HT_1$ receptor (5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{1C}$) and also 5-$HT_2$ (Hoyer et al. 1985) and 5-$HT_3$ (Kilpatrick et al. 1987) receptors in rat brain.

Selective agonists for 5-$HT_{1A}$ receptors cause hyperphagia in freely feeding rats, probably via activation of 5-$HT_{1A}$ autoreceptors on the raphe nuclei, as shown for 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) (Hutson et al. 1986). However, 8-OH-DPAT given at high dosage to food-deprived rats causes hypophagia which is explicable in terms of the high rate of feeding of the control animals and the behavioural disruption due to the 5-HT syndrome which also occurs (Dourish et al. 1985). Agonists with greater affinity for 5-$HT_{1B}$ receptors, i.e., RU24969, 1-[3-(trifluoromethyl) phenyl] piperazine (TFMPP) and 1-(-3-chlorophenyl) piperazine (mCPP), cause hypophagia and concomitant changes of motor activity when given systemically in both freely feeding (Kennett et al. 1987) and food-deprived rats (Kennett and Curzon 1988a). We now report that RU24969 and TFMPP but not 8-OH-DPAT cause hypophagia without changes of gross motor activity when infused into the PVN of food-deprived rats.

## Materials and methods

### *Animals*

Male Sprague-Dawley rats (180–220 g), Charles River, UK) were housed individually at 20 ± 1° C on a 12 h light-dark cycle (lights on 06:00 hours). Food, (diet 22F, Labsure, Poole, Dorset) and water were freely available unless stated otherwise.

### *Drugs*

RU24969 (Roussel UCLAF, Romainville, France), 8-OH-DPAT HBr and TFMPP HCl (Research Biochemicals Inc., Wayland, MA. USA) were dissolved in 0.9% NaCl.

### *Systemic administration of drugs*

Rats were deprived of food but not water for 18 h, given 0.9% NaCl, RU24969 (1 mg/kg), TFMPP (1 mg/kg) or 8-OH-DPAT (1 mg/kg) SC in a volume of 1 ml/kg and immediately replaced in their cages with a weighed amount of food pellets placed in the food hopper. After 30 min the remaining food was weighed and the amount eaten calculated. Experiments were performed between 12:00 and 14:00 hours.

### *Central administration of drugs*

*Surgery.* Rats were anaesthetised with pentobarbitone (Sagatal, May and Baker, 60 mg/kg IP) and a guide cannula (Plastic Products Ltd) implanted approximately 1 mm above the PVN of the hypothalamus using co-ordinates

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according to Paxinos and Watson (1982) as follows: A −1.5 mm, (bregma); L 0.3 mm; H 7.5 mm below dura. The guide was fitted with a dummy cannula (Plastic Products) and the animals transferred to perspex cages (30 × 30 × 30 cm).

*Experimental procedure.* Five days after surgery, food pellets but not water were removed from the cage and the bedding replaced. Eighteen hours later (between 12:00 and 14:00 hours) the dummy cannula was removed and an infusion cannula (Plastic Products) projecting 1 mm past the tip of the guide cannula was inserted into the PVN and connected via a length of PP10 polyethylene tubing to a peristaltic pump (Watson-Marlow).

Rats were infused at 1 µl/min for 1 min with RU24969 (0.5 µg, 1.0 µg, 2.0 µg), TFMPP (0.5 µg, 1.0 µg, 2.0 µg), 8-OH-DPAT (1 µg) or vehicle (0.9% NaCl). In a separate experiment, 8-OH-DPAT or vehicle were infused into freely feeding rats. The needle was left in place for a further 30 s to allow the infusate to diffuse away from it. A weighed amount of food pellets was placed on the floor of the cage and intake/30 min calculated as before.

Behaviour following infusion was recorded on videotape for subsequent counting of cage crossings. Approximately 3 days later, the procedure was repeated except that rats previously given 0.9% NaCl were given one of the 5-HT agonists (and vice versa). In one experiment, however, rats were given 0.9% NaCl, TFMPP (2.0 µg) and 8-OH-DPAT (1.0 µg) in random order.

*Histological verification of the infusion site*

After measuring food intake, rats were killed by an overdose of pentobarbitone and 1 µl of Indian ink infused at the injection site. Brains were removed, fixed in formal-saline and sectioned on a microtome. Animals were rejected from the study if the injection site was greater than 0.3 mm from the area of the PVN.

*Statistics*

Effects of subcutaneous injections were assessed by the Mann-Whitney *U*-test. Effects of PVN injections were assessed by 2-way ANOVA with repeated measures and when significant ($P<0.05$), multiple comparisons were made between 0.9% NaCl and drug-treated groups using the Wilcoxon matched pair signed rank test.

## Results

Table 1 shows that when rats previously deprived of food for 18 h were given RU24969 or TFMPP then food intake over the subsequent 30 min was significantly less than that of animals given 0.9% NaCl. This occurred following both subcutaneous and PVN injection.

RU24969 (0.5, 1.0, 2.0 µg) injected into the PVN, caused hypophagic responses which were dose dependent, with mean food intakes decreased by 17%, 30% and 70%, respectively. Comparable results were obtained for TFMPP (0.5, 1.0, 2.0 µg) with intakes decreased by 14%, 41% and 63%. The effects were significant at the two higher doses of both drugs, with 1 µg RU24969 and 2 µg TFMPP eliciting hypophagic effects comparable to those of 1 mg/kg of each drug given systemically. The above drug injections into the PVN did not significantly affect locomotion. Animals subsequently rejected on histological grounds did not show hypophagia. Although 8-OH-DPAT (1 mg/kg SC) was markedly hypophagic in food-deprived rats, 1 µg of the drug had no effect when infused into the PVN of both food deprived (see Table 1) and freely feeding animals (8-OH-DPAT, 1.7±0.7 g; 0.9% NaCl, 1.1±0.6 g; food/30 min, means±SEM, $n=5$/group, difference non-significant).

**Table 1.** Effects of RU24969 and TFMPP on food intake and locomotion in rats previously deprived of food for 18 h

| Treatment | | Food intake (g/30 min) | Cage crossings (no/30 min) |
|---|---|---|---|
| *Injected subcutaneously* | | | |
| 0.9% NaCl | | 4.2±0.2 (5) | n.d. |
| RU24969 | 1 mg/kg | 2.6±0.4 (6)* | n.d. |
| TFMPP | 1 mg/kg | 1.0±0.5 (5)*** | n.d. |
| 0.9% NaCl | | 5.1±0.3 | n.d. |
| 8-OH-DPAT | 1 mg/kg | 0.8±0.1*** | n.d. |
| *Injected into the PVN* | | | |
| 0.9% NaCl | | 4.7±0.8 (5) | 16±2 (5) |
| RU24969 | 0.5 µg | 3.9±0.5 (5) | 19±2 (5) |
| 0.9% NaCl | | 4.8±0.3 (11) | 13±2 (9) |
| RU24969 | 1.0 µg | 3.4±0.4 (11)** | 10±2 (9) |
| 0.9% NaCl | | 4.5±0.4 (6) | 20±3 (6) |
| RU24969 | 2.0 µg | 1.3±0.6 (6)* | 17±2 (6) |
| 0.9% NaCl | | 3.6±0.3 (5) | 16±2 (5) |
| TFMPP | 0.5 µg | 3.1±0.3 (5) | 21±2 (5) |
| 0.9% NaCl | | 4.6±0.4 (7) | 15±4 (7) |
| TFMPP | 1.0 µg | 2.7±0.5 (7)* | 12±3 (7) |
| 0.9% NaCl | | 4.3±0.5 (6) | 19±3 (6) |
| TFMPP | 2.0 µg | 1.6±0.4 (6)* | 18±2 (6) |
| 8-OH-DPAT | 1.0 µg | 4.8±0.5 (6) | 13±2 (6) |

No. of rats shown in brackets. Values are means±SEM
n.d. = not determined
Significances of differences from appropriate 0.9% NaCl-treated controls:
* $P<0.05$, ** $P<0.01$, *** $P<0.001$

## Discussion

RU24969 and TFMPP given systemically significantly reduced food intake by 18 h food-deprived rats, in agreement with previous results on freely feeding rats (Kennett and Curzon 1988a). The dose-dependent hypophagia which occurred (albeit at rather high dosage) when food-deprived rats were given the above 5-HT agonists by infusion into the PVN of the hypothalamus agrees with the similar effect of single doses of 5-HT and norfenfluramine (Shor-Posner et al. 1986).

RU24969 and TFMPP given systemically also cause hyperlocomotion (Kennett et al. 1987) and hypolocomotion (Kennett and Curzon 1988b), respectively. However, the hypophagic and locomotor effects appear to be separately mediated. Thus, RU24969 caused hypophagia without hyperlocomotion when injected into the PVN and it was previously shown that haloperidol prevented the hyperlocomotion but not the hypophagia caused by systemic injection of RU24969 (Kennett et al. 1987). The present study also indicates that TFMPP does not cause hypophagia merely as a result of decreased motor activity, as injection into

the PVN led to highly significant hypophagia without hypolocomotion.

The effects of drugs on the hypophagic response to systemic RU24969 (Kennett et al. 1987) indicate that it acts via postsynaptic 5-$HT_{1B}$ receptors and the present findings strongly suggest that these are located in the PVN. The receptors are hardly likely to be presynaptic, as activation would then tend to decrease 5-HT release. A resultant hypophagia seems improbable in view of the hypophagia caused by infusing 5-HT into the PVN (Shor-Posner et al. 1986).

The hypophagic effect of TFMPP on infusion into the PVN could be mediated by 5-$HT_{1C}$ receptors rather than 5-$HT_{1B}$ receptors, as mianserin, an antagonist with high affinity for 5-$HT_{1C}$ but not for 5-$HT_{1B}$ sites (Hoyer et al. 1985), opposed hypophagia caused by systemic TFMPP (Kennett and Curzon 1988a). 5-$HT_{1C}$ receptors may be important for the control of food intake in man, as Pazos et al. (1987) detected them in the human PVN but did not find 5-$HT_{1B}$ receptors in any region of the human brain. It is unlikely that 5-$HT_{1A}$ receptors in the PVN are involved in the control of feeding, as while the 5-$HT_{1A}$ agonist 8-OH-DPAT on systemic administration caused hypophagia in food-deprived rats, probably by disrupting feeding behaviour, it was without significant effect on feeding when infused into the PVN of either food-deprived or freely feeding animals.

The main conclusion from the present data and related studies is that RU24969 activates postsynaptic 5-$HT_{1B}$ receptors in the paraventricular nucleus of the rat hypothalamus to cause hypophagia. TFMPP has a similar effect on feeding, but this may also involve 5-$HT_{1C}$ receptors. These results imply that other hypophagic serotonergic drugs such as fenfluramine act (at least in part) by activating 5-$HT_{1B}$ or 5-$HT_{1C}$ receptors in the PVN. The hypophagic effects contrast with the hyperphagia which occurs in freely feeding rats on activation of 5-$HT_{1A}$ receptors in the raphe nuclei (Hutson et al. 1986).

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